43 ACETAMINOPHEN AND SALICYLIC ACID SCREEN AND QUANTITATION BY HPLC-DAD

43.1 Summary

43.1.1 Acetaminophen and salicylic acid are extracted from biological samples by making the samples slightly acidic with sodium acetate buffer and extracting with hexane/ethyl acetate. An aliquot of the extract is analyzed by high performance liquid chromatography-diode array detector (HPLC-DAD).

43.2 Specimen Requirements

43.2.1 0.5 mL blood, fluid or tissue homogenate.

43.3 Reagents and Standards

43.3.1 Acetaminophen, 1 mg/mL

43.3.2 Salicylic Acid, 1 mg/mL

43.3.3 Phenacetin, 1 mg/mL

43.3.4 Sodium acetate

43.3.5 Hexane

43.3.6 Ethyl acetate

43.3.7 Methanol

43.3.8 Glacial Acetic Acid

43.4 Solutions, Internal Standard, Calibrators and Controls

43.4.1 Sodium Acetate Buffer - weigh out 8.0 g sodium acetate, transfer to a 1 L volumetric flask and dissolve in approximately 800 mL deionized water. Adjust the pH to 4.5 with glacial acetic acid and QS to volume with deionized water.

43.4.2 Hexane/Ethyl acetate (50:50, v:v) - Mix 100 mL hexane with 100 mL ethyl acetate

43.4.3 Mobile Phase A (water with 0.2% glacial acetic acid) - In a 500 mL volumetric flask filled with approximately 450 mL of HPLC grade water, add 1 mL of glacial acetic acid. QS to volume with HPLC grade water. Filter before use.

43.4.4 Drug stock solutions:

43.4.4.1 If 1 mg/mL commercially prepared stock solutions are not available, prepare 1 mg/mL solutions from powders. Weigh 10 mg of the free drug, transfer to a 10 mL volumetric flask and QS to volume with methanol. Note: If using the salt form, determine the amount of the salt needed to equal 10 mg of the free drug, and weigh this amount. Stock solutions are stored capped in a refrigerator and are stable for 2 years.

43.4.5 The following are examples of acceptable procedures for the preparation of calibrators. Other quantitative dilutions may be acceptable to achieve similar results:

43.4.5.1 Working acetaminophen standard solution (0.5 mg/mL). Pipet 5 mL of 1 mg/mL acetaminophen stock solution into a 10 mL volumetric flask and QS to volume with methanol.

43.4.5.2 Working salicylic acid standard solution (0.25 mg/mL). Pipet 2.5 mL of 1 mg/mL salicylic acid stock solution into a 10 mL volumetric flask and QS to volume with methanol.

43.4.5.3 Working internal standard solution (50 µg/mL phenacetin): Pipet 500 µL of the 1 mg/mL stock solution of phenacetin into a 10 mL volumetric flask and QS to volume with methanol.

43.4.5.4 To prepare the calibration curve, pipet the following volumes of the 0.5 mg/mL acetaminophen stock solution and/or 0.25 mg/mL salicylic acid stock solution into appropriately labeled 16 x 125 mm screw cap test tubes. Evaporate to dryness under nitrogen. Add 0.5 mL blank blood to obtain the final concentrations listed below.

Amount of stock solution (µL)	Final concentration of acetaminophen (mg/L)	Final concentration of salicylic acid (mg/L)
400	400	200
200	200	100
100	100	50
60	60	30
40	40	20
20	20	10
10	10	5

43.4.6 Controls

43.4.6.1 Acetaminophen Control. Control may be from an external source or prepared in house using drugs from different manufacturers, lot numbers or prepared by a chemist different than the individual performing the extraction.

43.4.6.2 Salicylic Acid Control. Control may be from an external source or prepared in house using drugs from different manufacturers, lot numbers or prepared by a chemist different than the individual performing the extraction.

43.4.6.3 Negative control. Blood bank blood or equivalent determined not to contain acetaminophen or phenacetin.

43.5 Apparatus

43.5.1 Test tubes, 16 x 125 mm, round bottom, borosilicate glass with Teflon caps

43.5.2 Test tubes, 16 x 114 mm, glass centrifuge, conical bottom

43.5.3 Centrifuge capable of 2000-3000 rpm

43.5.4 Evaporator/concentrator

43.5.5 Vortex mixer

43.5.6 GC autosampler vials with inserts

43.5.7 pH meter

43.5.8 HPLC-DAD: Agilent Model 1100 HPLC-DAD

43.5.8.1 HPCL Instrument Conditions. The following instrument conditions may be modified to adjust or improve separation and sensitivity.

43.5.8.1.1 Elution Conditions

43.5.8.1.1.1	Column: Agilent Zorbax C8 150 mm x 4.6 mm, 5 µM particle size			
43.5.8.1.1.2	Column thermostat: 35° C			
43.5.8.1.1.3	Solvent A:	water with 0.2% glacial acetic acid		
43.5.8.1.1.4	Solvent B:	methanol		
43.5.8.1.1.5	Initial Flow Rate:	0.50 mL/min		
43.5.8.1.1.6	Injection vol.:	3µL with wash vial		
43.5.8.1.1.7	Stop time:	10.0 min with 7.0 min post time		
43.5.8.1.1.8	Gradient:	0-3.5 minutes	50% B	0.50 mL/min
		3.5-4 minutes	50% B	0.70 mL/min
		4-9 minutes	50% B	0.70 mL/min
		9-10 minutes	50% B	0.50 mL/min
43.5.8.1.1.9	Wavelength:	250 nm switch to 240 nm at 6.8 min		

43.6 Procedure

43.6.1 Label clean 16 x 125 mm screw cap tubes appropriately with calibrators, controls and case sample IDs.

43.6.2 Prepare calibrators and controls.

43.6.3 Add 0.5 mL case specimens to the appropriately labeled tubes.

43.6.4 Add 50 µL of the 50 µg/mL phenacetin internal standard working solution to each tube for a final concentration of 5 mg/L.

43.6.5 Add 1 mL sodium acetate buffer and 3 mL extraction solvent (50:50 hexane/ethyl acetate) to each tube.

43.6.6 Cap and rotate tubes for 30 minutes.

43.6.7 Centrifuge at approx 2500 rpm for 15 minutes. Transfer organic (upper) layer to appropriately labeled conical bottom test tubes.

43.6.8 Evaporate samples to dryness at approximately 60° C under nitrogen.

43.6.9 Reconstitute samples in 200 µL methanol. Transfer to GC autosampler vials for analysis.

43.7 Quality Control and Reporting

43.7.1 See Toxicology Quality Guidelines

43.8 References

43.8.1 M Kennedy, D Sullivan, R Steiner, and C Martinez, in house development

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